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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/835,287	04/13/2001	David R. Kaplan	071957-1102	4744

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EXAMINER	
GABEL, GAILENE	

ART UNIT	PAPER NUMBER
1641	12

DATE MAILED: 04/07/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/835,287	KAPLAN, DAVID R.	
	Examiner	Art Unit	
	Gailene R. Gabel	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for R ply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 February 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-20 and 37* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1-20 and 37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Amendment Entry

1. Applicant's amendment and response, filed 2/3/03, in Paper No. 11 is acknowledged and has been entered. Claims 21-36 have been cancelled. Claim 37 has been added. Currently, claims 1-20 and 37 are pending and are under examination.

Rejections Withdrawn

Claim Rejections - 35 USC § 103

2. In light of Applicant's argument, the rejection of claims 1-13 and 15-19 under 35 U.S.C. 103(a) as being unpatentable over Karkmann et al. (Journal of Immunological Methods, 1999) in view of Roth et al. (US 5,902,727) is hereby, withdrawn.

3. In light of Applicant's argument, the rejection of claims 1-20 under 35 U.S.C. 103(a) as being unpatentable over Lollini et al. (Immunological Blackboard, 1998) in view of Roth et al. (US 5,902,727) is hereby, withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 2, 6, 7, and 10 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 2, step b) has improper antecedent basis problem in reciting, "in cells". Change to "in said cells" for proper antecedent basis.

In amended claim 7, "fluoroscein" should be "fluorescein".

Claim 10, step i), lines 5-6, is confusing in reciting, "the deposition of tyramide", second occurrence. Please clarify.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. Claims 1-13, 15-19, and 37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Karkmann et al. (Journal of Immunological Methods, 1999) in view of McKinley et al. (Laboratory Investigation, (1991 Dec) 65 (6) 622-30 (Abstract)) or Merz et al. (Laboratory Investigation, (1995 Jul) 73 (1) 149-56 (Abstract)).

Karkmann et al. teach a method of detecting intracellular analyte, i.e. cytokines in tumor cells, in cells by flow cytometry using intracellular tyramine-based signal amplification technique (see Abstract and page 114, column 1). Karkmann et al. teach fixing peripheral blood mononuclear cells (PBMC) with formaldehyde, pemeabilizing the

cells with 0.5% saponin, and resuspending the cells in a buffer medium containing bovine serum albumin (BSA) and 0.5% saponin (see page 114, column 2 to page 115, column 1). Thereafter, Karkmann et al. teach staining the cells with fluorescein-labeled antibodies against the intracellular analyte which is linked to horseradish-peroxidase directly or indirectly by biotinylation, i.e. avidin-biotin, then adding tyramide substrate, wherein the peroxidase enzyme catalyzes the deposition of the tyramide in the cells comprising intracellular analyte. After the step of staining the cells with labeled antibodies, the cells are washed twice with saponin buffer to remove unbound binding partners (see page 115, column 2). Karkmann et al. specifically taught that while saponin is usually used as a permeabilization agent for intracellular staining, it is also capable of blocking peroxidase activity; thus, reducing nonspecific background staining or contamination staining. According to Karkmann et al., the tyramine-based signal amplification technique results in a 10 to 15 fold improvement of the signal compared to standard flow cytometric techniques using fluorescent label making it possible to detect even weakly stained cells (see page 116, column 2, pages 117 and 119).

Karkmann et al. differ from the instant invention in failing to disclose contacting the cells in a medium comprising chaotropic agent to wash the cells.

McKinley et al. teach that infectious scrapie prions are composed largely, of an abnormal isoform of the prion protein (PrP) designated PrPSc wherein PrPSc accumulates primarily intracellularly, i.e. within the cell cytoplasm, whereas cellular PrP (PrPC) is anchored to the external surface of the plasma. In determining subcellular localization of PrPSc, scrapie-infected cells are cultured, fixed, and then contacted with

a chaotropic agent, guanidine thiocyanate, before antibody staining and examination by electron microscopy. According to McKinley et al., intracellular PrPSc immunoreactivity was enhanced by denaturation with guanidine isothiocyanate which also permeabilized cells. The presence of PrPSc in secondary lysosomes is inferred from colocalization of guanidine isothiocyanate enhanced PrP immunoreactivity and acid phosphatase.

Merz et al. state that the molecular mechanism underlying tissue fixation in immunohistochemistry was not well understood but it is clear that available immunoreactive antigens are progressively lost during the fixation process. Merz et al., thus, teach use of a combination of an optimized antigen retrieval system and a powerful immunohistochemical staining protocol introducing a biotin amplification step, in which signal amplification is accomplished by covalent deposition of biotin molecules. The antigen retrieval system comprises pretreatment or contact of cells or tissue with detergent, protease, a chaotropic substance, or microwave heating which results in moderately improved immunostaining; however, biotinylated tyramine enhancement step proved to be the most efficient one, although the latter is not sufficient for many antigens when used without pretreatment or contacting step. Merz et al. teach that the combination of the antigen retrieval step with the biotinylated tyramine enhancement step resulted in a 100 to 10,000-fold boost in sensitivity without loss of specificity.

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to incorporate the teaching of McKinley or Merz in contacting the cells for detection of intracellular analyte to chaotropic agents such as guanidine thiocyanate, to the flow cytometric signal amplification method of detecting intracellular antigens as

taught by Karkmann because McKinley specifically taught that immunoreactivity of intracellular antigens is enhanced by denaturation with guanidine isothiocyanate; thus enhancing sensitivity in signal amplification detection of intracellular analyte, and Merz specifically further confirmed that addition of chaotropic substance to cells prior to biotinylated tyramine enhancement step results to improved immunostaining to as much as 100 to 10,000-fold boost in sensitivity without loss of specificity in detecting intracellular antigens.

Karkmann et al. and McKinley et al. or Merz et al. differ from the instant invention in failing to teach a signal provided by the method that is at least 20-fold and 50-fold greater than a signal by standard flow cytometry methods, as recited in claims 3-4.

However, the discovery of a degree of amplification signal of a known method, i.e. at least 20-fold and 50-fold greater than a signal by standard flow cytometry methods, are all result effective variables which the prior art references have shown may be obtained by optimization procedures. It has long been settled to be no more than routine experimentation for one of ordinary skill in the art to discover an optimum value of a result effective variable. "No invention is involved in discovering optimum ranges of a process by routine experimentation." Id. at 458, 105 USPQ at 236-237. The "discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art." Application of Boesch, 617 F.2d 272, 276, 205 USPQ 215, 218-219 (C.C.P.A. 1980). Since Applicant has not disclosed that the specific limitations recited in instant claims 3-4 are for any particular purpose or solve any stated problem and the prior art teaches that tyramide amplification methods often

vary according to various matrices used and parameters appear to work equally as well, absent unexpected results, it would have been obvious for one of ordinary skill to discover the optimum workable ranges of the methods disclosed by the prior art by normal optimization procedures.

6. Claims 1-20 and 37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lollini et al. (Immunological Blackboard, 1998) in view of McKinley et al. (Laboratory Investigation, (1991 Dec) 65 (6) 622-30 (Abstract)) or Merz et al. (Laboratory Investigation, (1995 Jul) 73 (1) 149-56 (Abstract)).

Lollini et al. teach a method and kit for detecting intracellular analyte, i.e. oncosuppressor protein p53, in osteosarcoma cells wherein flow cytometric detection is performed after tyramide signal amplification (see Abstract). Lollini et al. teach culturing the cells in fetal bovine albumin (FBS), harvesting the cells, fixing the cells with methanol, and completely pemeabilizing the cells with methanol or acetone. Thereafter, Lollini et al. resuspend the cells with analyte specific antibody, i.e. anti-p53, in a buffer medium containing PBS, BSA and TWEEN 20. After the step of staining the cells with labeled antibodies, the cells are further washed further with a medium containing PBS, BSA and TWEEN 20, to remove unbound binding partners from the suspension. In practice, Lollini et al. teach incorporating a primary antibody against the intracellular analyte into the cells, and then adding thereto a horseradish peroxidase-conjugated $F(ab')_2$ anti-mouse IgG. Thereafter, the cells are resuspended in fluorescein tyramide substrate so that the peroxidase enzyme catalyzes the deposition of tyramide in the

cells (see pages 1-2). According to Lollini et al., the tyramide signal amplification is an excellent system for the quantitative determination of intracellular antigens in cells by flow cytometry and is superior to standard flow cytometric assays, because of its capability to yield 10 to 15 stronger signal (see page 5).

Lollini et al. differ from the instant invention in failing to disclose contacting the cells in a medium comprising chaotropic agent to wash the cells.

McKinley et al. and Merz et al. have been discussed supra.

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to incorporate the teaching of McKinley or Merz in contacting the cells for detection of intracellular analyte to chaotropic agents such as guanidine thiocyanate, to the flow cytometric tyramide signal amplification method of detecting intracellular antigens as taught by Lollini because McKinley specifically taught that immunoreactivity of intracellular antigens is enhanced by denaturation with guanidine isothiocyanate; thus enhancing sensitivity in signal amplification detection of intracellular analyte, and Merz specifically further confirmed that addition of chaotropic substance to cells prior to biotinylated tyramine enhancement step results to improved immunostaining to as much as 100 to 10,000-fold boost in sensitivity without loss of specificity in detecting intracellular antigens.

Lollini et al. and McKinley et al. or Merz et al. differ from the instant invention in failing to teach a signal provided by the method that is at least 20-fold and 50-fold greater than a signal by standard flow cytometry methods, as recited in claims 3-4.

However, the discovery of a degree of amplification signal of a known method, i.e. at least 20-fold and 50-fold greater than a signal by standard flow cytometry methods, are all result effective variables which the prior art references have shown may be obtained by optimization procedures. It has long been settled to be no more than routine experimentation for one of ordinary skill in the art to discover an optimum value of a result effective variable. "No invention is involved in discovering optimum ranges of a process by routine experimentation." Id. at 458, 105 USPQ at 236-237. The "discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art." Application of Boesch, 617 F.2d 272, 276, 205 USPQ 215, 218-219 (C.C.P.A. 1980). Since Applicant has not disclosed that the specific limitations recited in instant claims 3-4 are for any particular purpose or solve any stated problem and the prior art teaches that tyramide amplification methods often vary according to various matrices used and parameters appear to work equally as well, absent unexpected results, it would have been obvious for one of ordinary skill to discover the optimum workable ranges of the methods disclosed by the prior art by normal optimization procedures.

Response to Arguments

7. Applicant's arguments with respect to claims 1-20 and 37 have been considered but are moot in view of the new grounds of rejection.

8. No claims are allowed.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gailene R Gabel whose telephone number is (703) 305-0807. The examiner can normally be reached on Monday-Thursday 6:00 AM to 3:30 PM and alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long V. Le can be reached on (703) 305-3399. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Gailene R. Gabel
April 6, 2003

LONG V. LE
SUPERVISORY PATENT EXAMINER
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04/06/03